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Comprehensive Analysis of RP-HPLC Method Development and Validation for Metformin and Empagliflozin Stability

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ABSTRACT

Metformin's mechanisms of action are distinct from other classes of oral antihyperglycemic drugs. It primarily reduces blood glucose levels by inhibiting hepatic glucose production (gluconeogenesis) and enhancing insulin sensitivity. Metformin HCl and empagliflozin are oral antidiabetic agents that synergistically manage blood glucose levels.

A novel, precise, rapid, accurate, sensitive, specific, and stable reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous quantification of Metformin and Empagliflozin in bulk and dosage forms. This newly developed RP-HPLC method offers advantages over traditional reversed-phase HPLC, including reduced solvent consumption, shorter retention times, improved resolution, and lower operational costs. The chromatographic separation was achieved using a Waters HPLC system equipped with a photodiode array (PDA) detector and an autosampler. Both Metformin and Empagliflozin were subjected to stress conditions such as acidic, alkaline, oxidative, thermal, and photodegradation environments. Significant degradation was observed under acidic conditions. The newly developed RP-HPLC method was rigorously validated for system suitability, linearity, robustness, accuracy, and precision, ensuring its reliability for routine analysis of these antidiabetic medications.

Keywords: RP-HPLC; Metformin; Empagliflozin; Stability-indicating method; Tablet dosage form

I. INTRODUCTION[1-4]

Metformin's mechanisms of action are distinct from other classes of oral antihyperglycemic drugs. It primarily reduces blood glucose levels by inhibiting hepatic glucose production (gluconeogenesis), decreasing intestinal glucose absorption, and enhancing insulin sensitivity by increasing peripheral glucose uptake and utilization. It is well established that metformin inhibits mitochondrial complex I activity, which is generally postulated to be the primary mechanism behind its potent antidiabetic effects. These processes collectively lead to a decrease in blood glucose levels, effectively managing type II diabetes and improving glycemic control.

Empagliflozin, on the other hand, is used alongside diet and exercise, and sometimes with other medications, to lower blood sugar levels in individuals with type 2 diabetes. It is also prescribed to reduce the risk of stroke, heart attack, or death in people with type 2 diabetes who have heart and blood vessel disease. Additionally, empagliflozin is used in adults with heart failure to reduce the risk of hospitalization and death due to heart and blood vessel disease. Empagliflozin belongs to a class of medications known as sodium-glucose co-transporter 2 (SGLT2) inhibitors. It lowers blood sugar by promoting the excretion of glucose through the urine. However, empagliflozin is not used to treat type 1 diabetes or diabetic ketoacidosis, a serious condition that can develop if high blood sugar is not treated.

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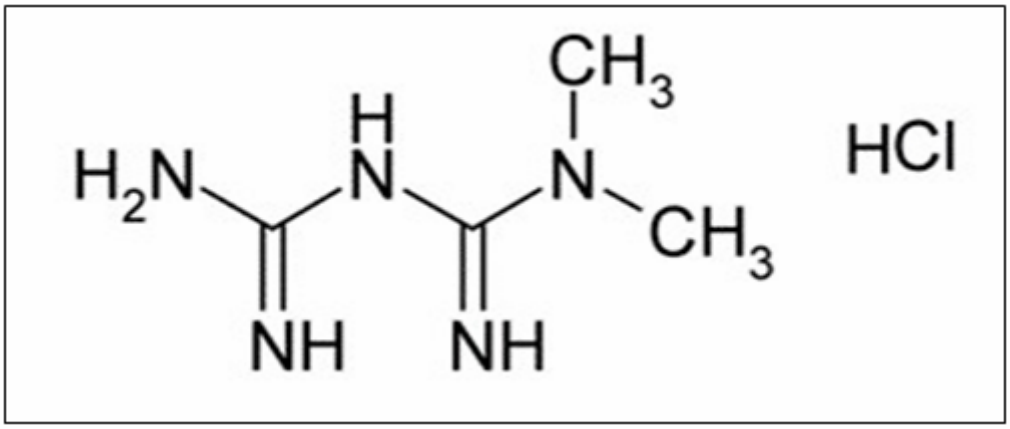

Figure 1 Chemical Structure of Metformin

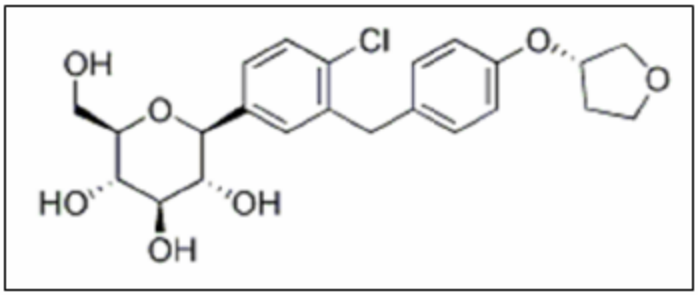

Figure 2 Chemical Structure of empagliflozin

III. MATERIAL AND METHODS

API gift samples of metformin hydrochloride and empagliflozin were provided by Spectrum Pharma Research Solutions, Hyderabad. HPLC grade Acetonitrile, water, and other chemicals from Rankem, Hyderabad. Waters HPLC 2695 SYSTEM is an integrated Autosampler with Quad Pump, Photo Diode Array Detector, and Empower 2 Software. Spectrophotometer T60, 2 mm and 10 mm broadband with quartz cells compatible with UV win 6 were used to measure the absorbance of Metformin and Empagliflozin solutions.

2.1.Chemicals and Reagents

Potassium dihydrogen phosphate, acetonitrile, HPLC grade, HPLC grade water, Trimethylamine is used. Performance standards for metformin and empagliflozin. Trijardy XR tablets containing 1000 mg metformin and 10 mg Empagliflozin were purchased.

2.2.Preparation of standard solution

The exact weight is 125 mg of metformin HCl (API) and 12.5 mg of empagliflozin (API), separately filled into a 25 ml volumetric flask and dried with 100 ml. Add 3/4 of the mixture to both of these flasks, sonicate for 10 minutes, and finally label with diluent. The resulting concentrations were 5000 µg/ml metformin and 125 µg/ml empagliflozin.

2.3.Preparation of 0.1% Orth phosphoric Acid

Accurately pipette 1.0 mL of OPA into a clean & dried 1000 mL volumetric flask, add 100 mL of milli-Q water, stir well, and finally makeup up the mark with milli-Q water.

2.4.Preparation of Mobile Phase

It consisting a mixture of 0.1%OPA and Acetonitrile at a ratio of 50:50 v/v. Preparation of Diluent: It is a mixture of Acetonitrile and milli-Q water at a ratio of 50:50 v/v.

2.5.Preparation of Standard Working Solutions (100% solution)

Pipette 1 ml from each stock solution and transferred into a clean and dried 10 ml volumetric flask and finally makeup to the mark with diluent. The resultant concentrations are 500 µg/ml of metformin and 12.5 µg/ml of empagliflozin.

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2.6.Preparation of Sample Stock Solutions

10 tablets are randomly selected and weighed and the average weight of each tablet is calculated, all tablets were grounded into fine powder. The weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, add 50 ml diluent, sonicated for 25 minutes and finally make up to the mark with diluent. All the content was passed through 0.45 µm filter paper. The resultant concentration was 5000 µg/ml of metformin and 125 µg/ml of empagliflozin.

2.7.Preparation of Sample Working Solution (100% solution)

Pipette 1 ml of filtered sample stock solution; transfer it into a 10 ml volumetric flask and make up to the mark with diluent. The resultant concentrations were 500 µg/ml of metformin and 12.5 µg/ml of empagliflozin.

2.8.Optimized Chromatographic Method

The separation of Metformin Hydrochloride and Empagliflozin was achieved on a Kromosil C18 column (250x4.6 mm; 5.6 µ) and eluting with a mobile phase consisting of a 50:50 v/v mixture of Acetonitrile and Buffer [0.1% orthophosphoric Acid (pH 2.8)] at a flow rate of 1.0 mL/min. The analytes were monitored at 260 nm. The injection volume was 10 µl. The total run time for the elution of the compound was 6 min.

- **Column:** Kromasil C18; 50 x 4.6 mm; 5
- **Column temperature:** 30°C
- **Flow rate:** 1 mL/min Lµ
- **Injection volume:** 10
- **Detector wavelength:** 260 nm
- **Run time:** 6 min

2.9.Instrumentation

Chromatographic separation was achieved in a UPLC water purification system equipped with a built-in autosampler and PDA detector. The processing of the selected components is carried out using Empower 2 software. A hot air oven is used for the thermal degradation of the sample, and a UV cutting machine and a model 23400 UV camera equipped with a UV fluorescent lamp with a wavelength between 200 and 300 nm are selected for photolytic degradation. Ultrasonic bath (Toshcon by Toshconiv), digital pH meter (Adwa - AD 1020), and UV-Visible spectrophotometer (Labindia UV 3000) were used in the study[6]

IV. RESULTS & DISCUSSION

3.1.Method validation[7]

The US Pharmacopeia (USP) and US Food and Drug Administration (FDA) both refer to ICH guidelines. The most widely applied validation characteristics are accuracy, precision, specificity, linearity, range, robustness, the limit of detection, the limit of quantification, the limit of detection, and the limit quantification.

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3.2.System Suitability

Table 1 System suitability parameters

Drug	Retention time	Area	USP plat count	USP tailing
Metformin HCl	2.192	9877896	4354	1.33
Empagliflozin	3.200	954988	7073	1.48

It is the checking of a system to ensure system performance before or during the analysis of the unknown. It tests are an integral part of the chromatographic method and are used to verify that the resolution & reproducibility of the system are adequate for the analysis to be performed. In this, plate count (N), tailing factor (T), resolution (Rs), and reproducibility (%RSD) are determined from the replicate injection of the standard. The acceptable limit of %RSD is less than 2%. [Table 1].

3.3.Specificity

The method can accurately measure the analyte response in the presence of all potential sample components. In this study, the method was evaluated by injecting 10 µl of blank sample and placebo into HPLC. [Fig No. 3, 4, 5 & 6].

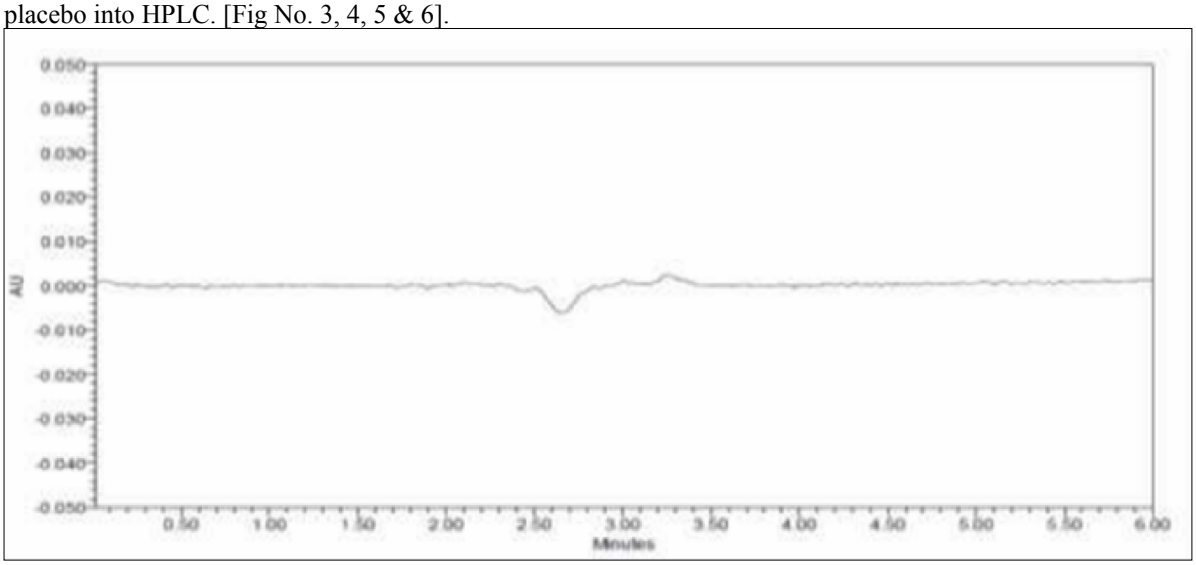

Figure 3 Typical chromatogram of blank

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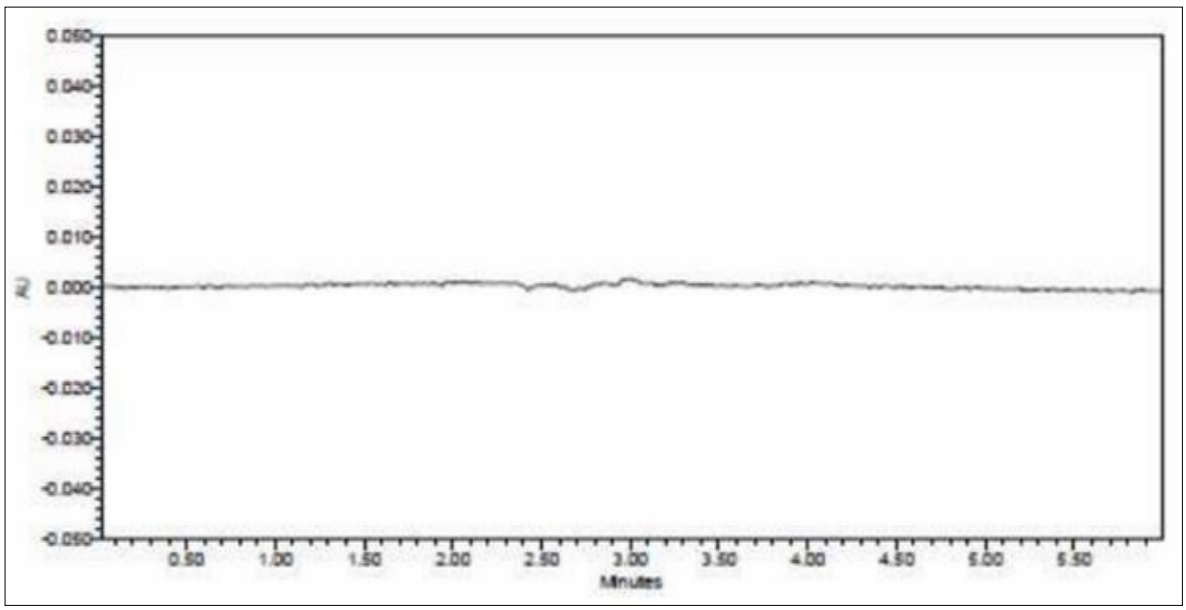

Figure 4 Typical Chromatogram of Placebo

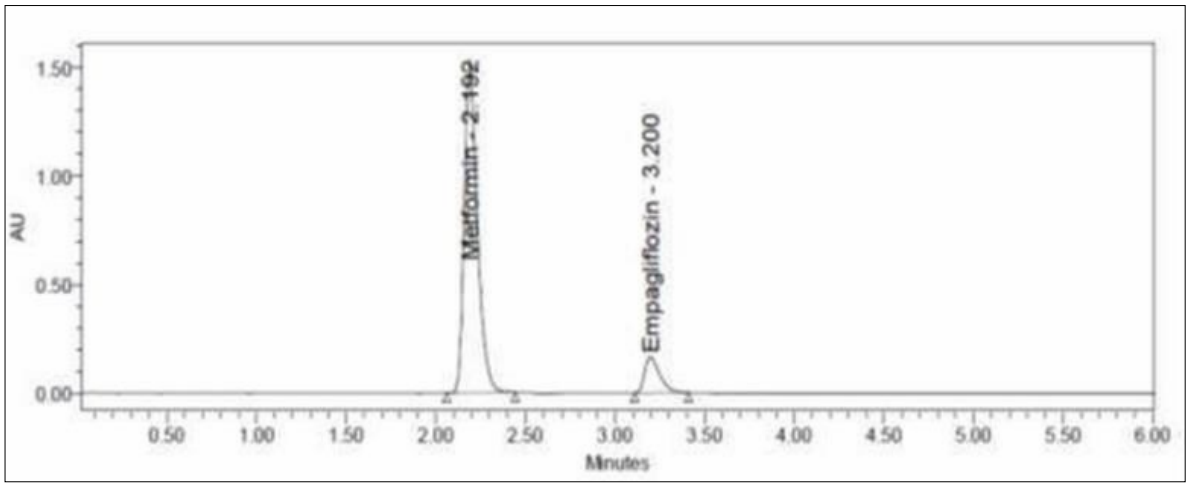

Figure 5 Standard chromatogram of metformin HCL and Empagliflozin

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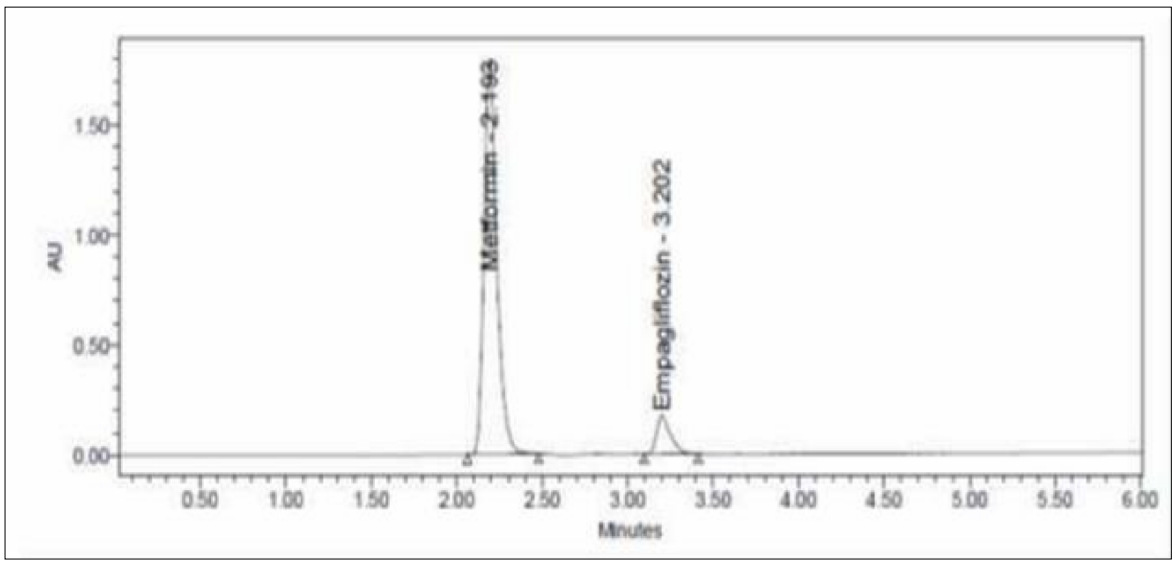

Figure 6 Typical chromatogram of a sample (Tablet Dosage Form)

Table 2 Recovery studies of metformin HCL and Empagliflozin

Drug	Level of spike solution	Amount present (mg/mL)	Amount added	Amount recovered	% Recovery	% RSD
Metformin HCL	50 %	500	250	249.82	99.93	0.73
	50 %	500	250	254.81	101.93	
	50 %	500	250	250.17	100.07	
	100 %	500	500	500.01	100	
	100 %	500	500	498.60	99.72	
	100 %	500	500	502.45	100.49	
	150 %	500	750	760.42	101.39	
	150 %	500	750	754.75	100.63	
	150 %	500	750	755.90	100.79	
Empagliflozin	50 %	12.5	6.25	6.20	99.29	0.75
	50 %	12.5	6.25	6.32	101.26	
	50 %	12.5	6.25	6.30	100.92	
	100 %	12.5	12.5	12.69	101.54	
	100 %	12.5	12.5	12.60	100.87	
	100 %	12.5	12.5	12.58	100.71	
	150 %	12.5	18.75	18.66	99.55	
	150 %	12.5	18.75	18.83	100.46	
	150 %	12.5	18.75	18.76	100.67	

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3.4.Accuracy

The accuracy of this method is evaluated by the standard addition method. A certain number of standard data is added to the number of standard solutions at three different levels. Solutions were analyzed for mean recovery and %RSD.

Studies were conducted for metformin and empagliflozin at three different concentrations of 50%, 100%, and 150%. 10 µL HPLC and % recovery and % RSD were injected. Shown in Table 2 [5].

3.5.Precision

Precision is the degree of agreement between individual test results when an analytical method is used several times to obtain several samples from the same sample. Precision is defined as reproducible precision and is studied for accuracy and intraday precision of the method with six injections of 10 µL and the peak area of duplicate injections[5].

3.6.Detection and Qualification limits

According to the Pharmacopoeial Guidelines (USP, 2011), the detection limit is defined as a concentration with a signalto-noise ratio of 3:1, with a calculated ratio for the quantitative limit of 10:1. The detection value for metformin and empagliflozin are 0.01 and 0, respectively. .01 ppm. The limits of quantitation for metformin and empagliflozin were 0.04 and 0.02 ppm, respectively. (fig.no 7 & 8)[8].

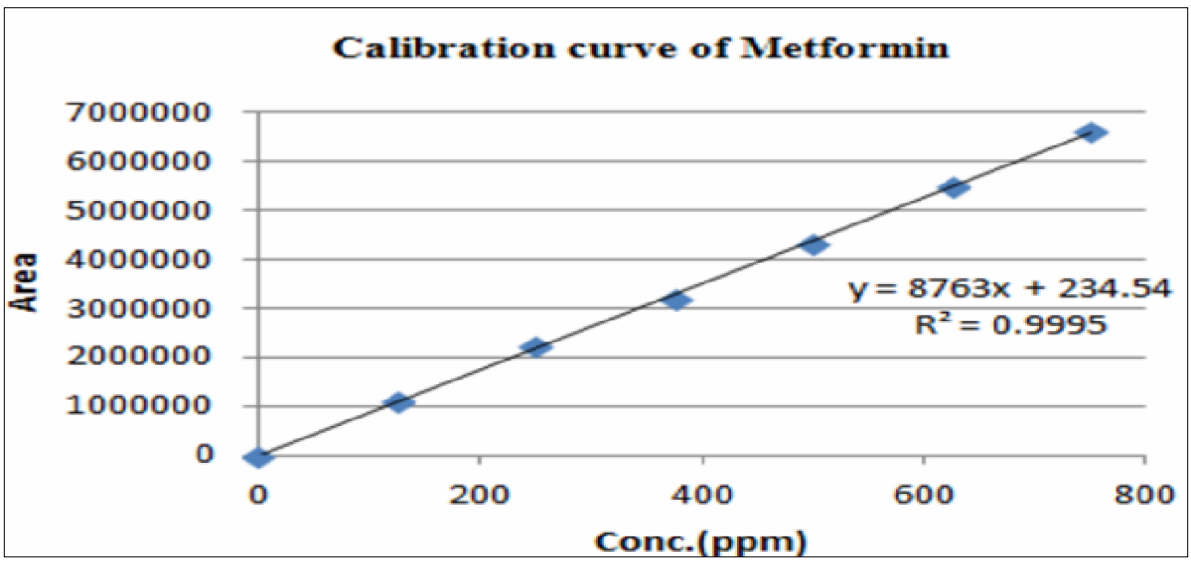

Figure 7 Linearity for Metformin

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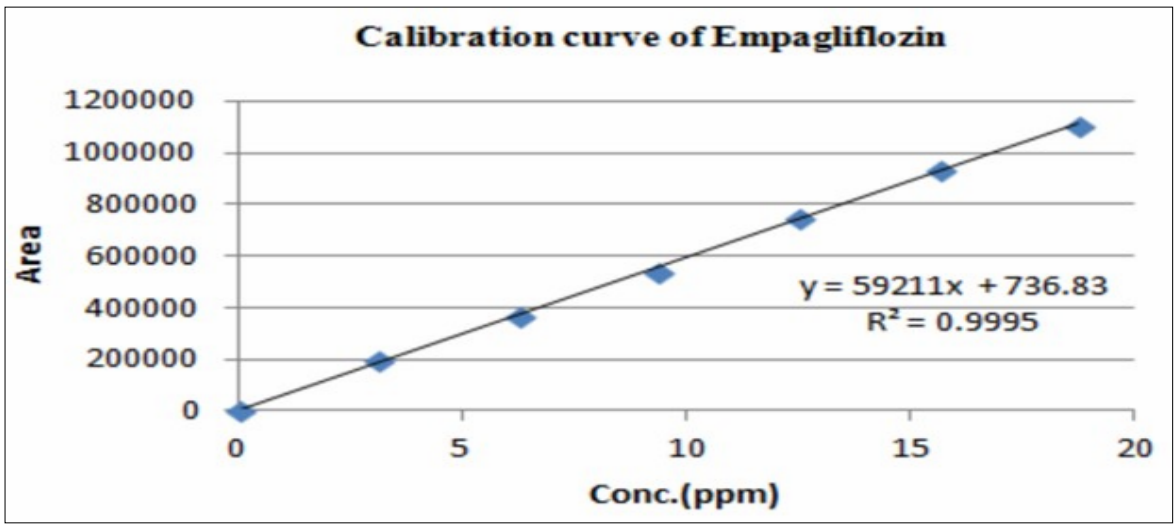

Figure 8 Linearity for Empagliflozin

3.7.Robustness

It is the capacity of a method to remain unaffected by small, deliberate variations in method parameters. It was indicated by changing the flow rate, mobile phase composition, and temperature.

3.8.Limit of Detection (LOD) & Limit of Quantification (LOQ)

LOD is the lowest concentration of an analyte in a sample that can be detected. LOQ is the lowest concentration of an analyte in a sample that can be quantized. The LOD and LOQ of metformin and empagliflozin were determined from a standard deviation of the response and the slope.

3.9.Assay Procedure

The assay was performed by the marked product (Synjardy XR 12.5 mg/500 mg of metformin and empagliflozin. The prepared sample and standard solution were injected into HPLC and peak areas were recorded. Finally, the percentage amount of the drug was calculated.

3.10.Degradation studies

Forced degradation involves exposing new drug substances and products to conditions more severe than accelerated conditions to study their chemical behavior. This information aids in the development of formulations and packaging. In this study, forced degradation of Metformin and Empagliflozin standard solutions was conducted under various conditions: oxidation, acidic, alkaline, dry heat, photostability, and neutral degradation.

3.10.1. Oxidation

1 ml of the standard stock solution of Metformin and Empagliflozin was pipetted into separate volumetric flasks. To each flask, 1 ml of 20% hydrogen peroxide (H O) was added. The solutions were maintained at 60°C for 30 minutes. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml, respectively. A 10 µl aliquot was injected into the system, and chromatograms were recorded to evaluate sample stability.

3.10.2. Acid Degradation Studies

1 ml of the stock solution of Metformin and Empagliflozin was pipetted into separate volumetric flasks. To each flask, 1 ml of 2N hydrochloric acid (HCl) was added, and the mixtures were refluxed at 60°C for 30 minutes. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml, respectively. A 10 µl aliquot was injected into the system, and chromatograms were recorded to evaluate sample stability.

3.10.3. Alkali Degradation Studies

1 ml of the stock solution of Metformin and Empagliflozin was pipetted into separate volumetric flasks. To each flask, 1 ml of 2N sodium hydroxide (NaOH) was added, and the mixtures were refluxed at 60°C for 30 minutes. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml,

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respectively. A 10 µl aliquot was injected into the system, and chromatograms were recorded to evaluate sample stability.

3.10.4. Dry Heat Degradation Studies

The standard drug solutions were placed in an oven at 105°C for 6 hours. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml. A 10 µl aliquot was injected into the system, and chromatograms were recorded to assess the stability of the samples.

3.10.5. Photostability Studies

The photochemical stability of the drug was studied by exposing the stock solutions to UV light in a UV chamber for 7 days or 200-watt hours/m² in a photostability chamber. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml. A 10 µl aliquot was injected into the system, and chromatograms were recorded to assess the stability of the samples.

3.10.6. Neutral Degradation Studies

Stress testing under neutral conditions was conducted by refluxing the drug in water for 6 hours at 60°C. The resultant solutions were diluted to achieve concentrations of 500 µg/ml and 12.5 µg/ml. A 10 µl aliquot was injected into the system, and chromatograms were recorded to assess the stability of the samples.

V. DISCUSSION

The research presented in this review involves the development and validation of a new RP-HPLC method for the simultaneous assay of Metformin and Empagliflozin. The method development included the selection of an optimized wavelength, which was determined to be 226 nm. Various mobile phases and columns were tested to optimize the RP-HPLC method.

A new isocratic RP-UPLC-DAD method was developed and validated for the determination of Metformin and Empagliflozin in bulk and tablet forms. The method was evaluated for precision, specificity, limit of quantitation (LOQ), limit of detection (LOD), linearity, and robustness.

VI. CONCLUSION

The developed method is simple, precise, accurate, and reliable for the simultaneous estimation of Metformin HCL and Empagliflozin in combined doses, adhering to ICH guidelines for stability. The %RSD of all results is less than 2-5%, indicating a high level of precision. The proposed method demonstrated good accuracy, linearity, and precision in the determination of the two drugs in laboratory-prepared mixtures with varying doses, making it suitable for quality control purposes.

REFERENCES

[1]. De B; Sen S and Chakraborty R. Diabetes Mellitus Analysis and Advancement; 1st Edition;CBS Publishers & Distributors, India, 2008, pp 105-124.
[2]. Diabetes Mellitus; Central Council for Research in Homoepathy; 1st Edition; Central Chinmaya Mission Trust, India, 2010, pp 96-118.
[3]. Banerjee S.Oral Anti-diabetics - Current Concepts; 3rd Edition;Scientific Publishing, New Delhi, India, 2018, pp 221-247.
[4]. Tripathi KD. Pharmacological Classification of Drugs; 6th Edition, Jaypee Brothers Medical Publishers, India, 2021, pp 116-131.
[5]. Vinay Kumar D and J. V. L. N. Seshagiri Rao, "A New Validated Stability Indicating RP-HPLC Method for Simutaneous Estimation of Metformin Hydrochloride and Empagliflozin in Tablet Dosage Forms," IRJPMS, Volume 1, Issue 5, pp. 16-22, 2018.
[6]. Padmaja, N., Veerabhadram, G. Development and validation of an analytical method for Simultaneous estimation of Empagliflozin and Linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Der Pharmacia Lettre. 2015; 7(12), 306-12.
[7]. Nachiket, S.D., Ganesh, S.S., Jyoti, J.V. Simultaneous Estimation, Validation and Force Degradation Study of Metformin Hydrochloride and Empagliflozin by RP-HPLC Method.Research J Science and Tech. 2019; 11(2), 1-5.
[8]. ICH Topic Q2 (R1)-Validation of Analytical Procedures: Text and Methodology, European Medicinal Agency, pp. 1-15, 1995.
[9]. Shyamala, Nirmala K, Mounika J and Nandini B: Der Pharmacia Lettre 2016; 8(2): 457-464.
[10].

http://www.scholarsresearchlibrary.com/a

bstract/validatedstabilityindicating-rphplc-method-fordetermination-ofempagliflozin-6371.html

[11]. Padmaja N and Veerabhadram G: Der Pharmacia Lettre 2015; 7(12): 306-312.

[12]. Ayoub and Bassam M: UPLC simultaneous determination of empagliflozin, linagliptin, and metformin. RSC Advances 2015; 5: 116.